Characterization of Texture and Mechanical Properties of Heat-Induced Soy Protein Gels

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Heat-set gels were prepared from acid-precipitated soybean proteins at various heating temperatures (80–100°C), protein concentrations (18–20%), and proportions of glycinin. The gels were evaluated for mechanical parameters by means of a compression-decompression test. Gels formed at higher heating temperature and protein concentration were firm, tough and unfracturable. The elasticities of the gels were similar at all protein concentrations and were lower when heated at higher temperature. Heating above 93°C was necessary for formation of rigid gels. The glycinin/β-conglycinin ratio affected the texture of the gels. Three-dimensional representation of the gels through factor analysis of instrumental data and calculation of factor scores was useful to evaluate the texture of the gels.

KEY WORDS: Compression test, factor analysis, gel properties, gel texture, mechanical parameters, soy protein, soy protein gel, statistical analysis, thermal gelation, three-dimensional representation.

Soy proteins play important roles in many foodstuffs because of their nutritional value and their contribution to food texture. In order to make optimal use of soy proteins as functional ingredients, we need better insight into the effects of processing conditions on gel properties. In particular, because the ability to form a gel contributes to creation of texture in food systems, the effects of heat on protein solutions at high concentration needs to be studied in greater detail.

Gels are characterized by relatively high viscosity, plasticity, and elasticity (1). In the case of soy proteins, heating above 60°C is necessary to induce dissociation of the quaternary structure of globulins and to cause unfolding of the protein subunits and a consequential increase in viscosity (2,3). Soybean proteins consist of two major components, β-conglycinin and glycinin. These two globulins have different structures and molecular properties, possess different abilities to gel, and have different gel properties (4–7). It has been demonstrated that glycinin gels are firmer and more elastic than β-conglycinin gels (6,8). Differences in thermal stability of glycinin and β-conglycinin, especially in the presence of various salt concentrations, may provide gels with different physical properties. It has also been demonstrated that heat-induced gelation of aqueous dispersions of soy globulins is affected by protein concentration, and time and temperature of heating (2,9,10). Thus, one can expect to change the physical properties of soy protein gels by changing the gelling conditions. However, lack of information on textural properties of soy protein gels and/or relationships between texture and physical properties of the gels limit application of gelling conditions in food usage of soy proteins.

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Analysis and evaluation of food texture are often done by measuring physical properties, such as compression and penetration tests. For food gels, the forces required for various degrees of compression and penetration have been measured to characterize physical properties and texture (11–13). In this study, we attempted to characterize mechanical properties and texture of soy protein gels made with various gelling conditions. The effects of temperature, protein concentration, and ratio of glycinin to β-conglycinin on gel properties were investigated at relatively high protein concentrations (18–20%) by measuring various mechanical parameters.

MATERIALS AND METHODS

Materials. Defatted, low heat-treated soybean meal was donated by Ajinomoto Co. Inc. (Tokyo, Japan). Protein solubility (NSI) and protein and fat contents of the defatted soybean meal were 85, 53, and 0.6%, respectively. The acid precipitated soy protein (APP) and the β-conglycinin-rich fraction were prepared from the soy meal according to the method of Thanh (14). No further purification was attempted. NSI, protein, and fat contents of the APP were 94, 92.8, and <0.1%, respectively.

Determination of 2S globulin, β-conglycinin and glycinin proportions. The protein solution was centrifuged at 20°C in 12 mL of 10–30% (w/v) linear sucrose gradient in 35 mM potassium phosphate buffer (pH 7.6) containing 0.4M NaCl, 10 mM 2-mercaptoethanol and 0.02% NaN_3 at 248,850 × *g* for 17.5 hr in a Hitachi (Tokyo, Japan) RPS 40T rotor. After centrifugation, the gradient was divided into 0.4-mL fractions and measured at 280 nm simultaneously with an ISCO density gradient fractionator (Isco, Inc., Lincoln, NB). Protein content of each fraction was measured using the method of Lowry (15), and glycinin and β-conglycinin contents were determined. The APP consisted of 20.9% 2S proteins, 23.2% β-conglycinin, and 55.9% glycinin, which gave a glycinin/β-conglycinin ratio of 2.41. The β-conglycinin-rich fraction was composed of 28% 2S proteins, 48% β-conglycinin, and 24% glycinin.

Preparation of gels. Heat-set soy protein gels in concentrations of 18–20% protein were prepared according to the following procedures. The APP was suspended in 35 mM potassium phosphate buffer (pH 7.6) containing 0.4M NaCl with stirring. The suspensions were adjusted to pH 7.6 by adding 2N NaOH dropwise, and then was mixed well with a blender for 10 min. After deaeration, the mixture was poured into a 2 mm gap between two glass plates equipped with a silicon spacer (2 mm thickness) for sealing, and heated at different temperatures (80–100°C) for 30 min. The gel sheets were removed and cut into 2 cm squares. To lower the glycinin/β-conglycinin ratio, 65.9 g of the β-conglycinin-rich fraction was added to 34.1 g of the APP. Thus, the mixture (25.5% 2S proteins, 39.6% β-conglycinin, and 34.9% glycinin) gave a ratio of 0.88, except that the proportion of 2S proteins was increased slightly. This mixture was referred to as

low glycinin-APP. Gels at a concentration of 20% were prepared from the low glycinin-APP as described above.

Measurement of mechanical properties of gels. A uniaxial compression-decompression test was carried out using a KES-FB 3 Compression Tester (Kato Tech Co. Ltd., Japan) equipped with a cylindrical plunger with a cross-sectional area of 0.25 cm^2. The tests were performed at 20°C. The plunger descended at a rate of 1.2 mm per min. The measurement was done by compressing the sample until rupture, and then the direction was reversed to move upward at the same speed. The plunger was reversed to the point where there was an increase in deformation with a decrease in force, denoted as R in Figure 1. This compression-decompression test gave force deformation curves, as shown in Figure 1. Compression work (CW) and decompression work (DW) were determined as the area under the compression and decompression curves, respectively. Resiliency (RS) was calculated from the equation:

$$RS\ (\%) = (DW/CW) \times 100 \quad [1]$$

Compressibility (CM) at rupture was measured as percent deformation using the equation:

$$CM\ (\%) = [\text{absolute deformation (Dr)/sample thickness}] \times 100 \quad [2]$$

Force (F) at rupture was determined as the force at yield which was characterized by the maximum, R, in the compression curve tracing as shown in Figure 1. The compression-decompression tests were then carried out at different levels of compression until rupture, i.e., at small (S), medium (M), and large (L) compression levels as shown in Figure 1. The forces were set at 15, 30, and 60% at rupture force for small, medium, and large compression levels, respectively. In the tests, the plunger returned automatically at the forces provided. From these tests, the parameters of CW, RS, CM, and F at different levels of compression were determined. The measurement was repeated 5–10 times with the gel samples. The data were reproducible to about ±5% on repeated runs.

Statistical analysis. Statistical differences were determined by using an analysis of variance in conjunction with a Duncan's Multiple Range Test and a Bonferroni's Multiple Comparison Test. This was done using an NEC personal computer and High Quality Analysis Libraries for Business and Academic Users (HALBAU) software package. Data was also subjected to factor analysis (principal factor method) using a multivariate analysis program (Microsystems Co., Ltd., Japan). The data was composed of the values taken for 16 variables: CW, RS, CM, and F at small, medium, and large compression levels and at rupture for the 24 gel samples examined.

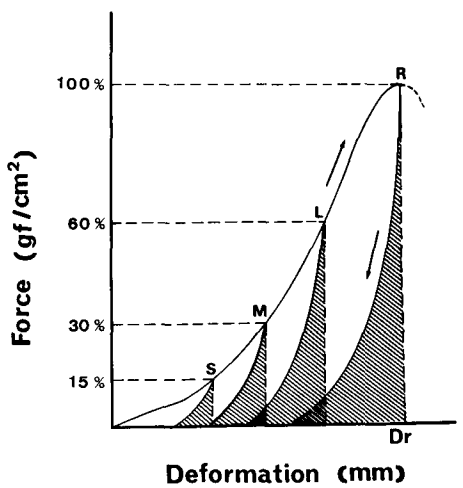

FIG. 1. Generalized force-deformation tracing of the compression-decompression test. R denotes rupture point; Dr, absolute deformation at rupture; L, M, and S, large, medium, and small compression levels, respectively.

RESULTS AND DISCUSSION

Effects of heating temperature and protein concentration on mechanical properties of APP gels. Changes in mechanical properties of APP gels are illustrated in Figure 2, where the plots are based on the means and 95% confidence intervals are not indicated because of small variation. Changes in rupture force of gels formed at different temperatures from 18, 19, and 20% APP solutions are shown in Figure 2A. The rupture force increased with elevation of heating temperature and protein concentration. A significant increase was observed over the temperature range of 80–100°C in the case of the 20% APP gel ($p < 0.05$). Similar increases were observed in both the 18 and 19% APPs gels, except that there were no significant increases from 85 to 90°C (0.05 level). Differences between protein concentrations became apparent above 93°C. The compression work required to cause rupture of the gels increased with heating temperature and protein concentration similar to the case of rupture force (Fig. 2B). The marked increases in rupture force and compression work with increasing heating temperature above 93°C suggest that the formation of new and substantial network structure occurred within the gel at those temperatures. Glycinin and β-conglycinin exhibit apparent denaturation temperatures of 90°C and 75°C, respectively (16). Therefore, the structure formation above 93°C, demonstrated in results of Figure 2A and 2B, may be attributed to conformational changes followed by association of glycinin molecules. In other words, glycinin may play a significant role in development of the gel network of APP above 93°C. Changes in compressibility at rupture of the gels are shown in Figure 2C. Gel compressibility increased with increased heating temperature and protein concentration similar to the case of rupture force and compression work, except that there were no significant increases from 85 to 93°C in the gels from 18% APP. Differences between the protein concentrations became apparent above 93°C. Resiliency decreased significantly with increased heating temperature ($p<0.05$). This decrease was gradual, as shown in Figure 2D. Differences between protein concentrations were not substantial. In contrast to rupture force, compression work and compressibility, resiliency changed gradually over the temperature range below the denaturation temperature of glycinin. This suggests some contribution of the β-conglycinin to gel structure manifested in resiliency.

Figure 2 shows that the mechanical properties of soy protein gels formed by heating may differ depending on protein concentration and heating temperature. On the other hand, the mechanical parameters of rupture force, compression work, and resiliency correspond to hardness, toughness, and elasticity, respectively (17–19).

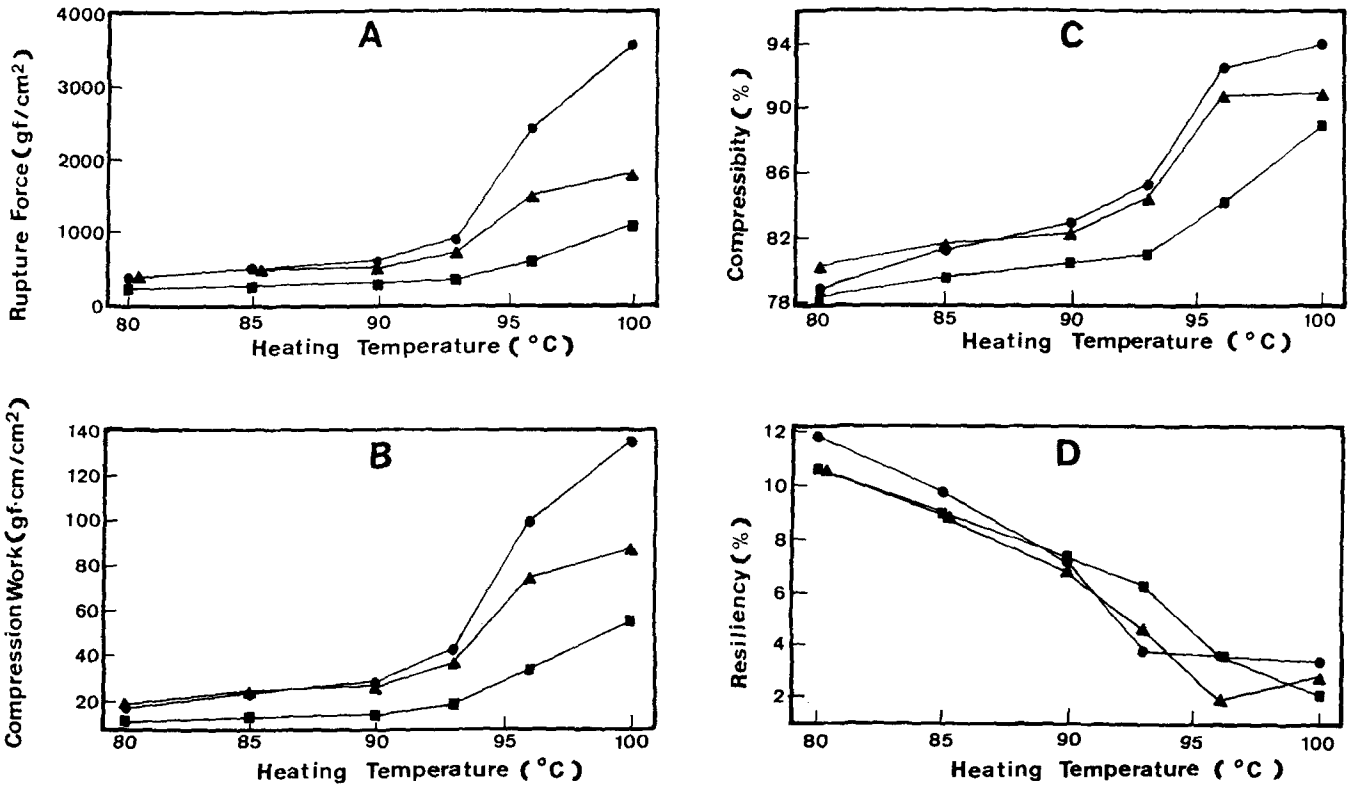

FIG. 2. Effects of heating temperature and protein concentration on gel properties. A denotes rupture force; B, compression work; C, compressibility; and D, resiliency. Protein concentrations of the APP used were 18% (■), 19% (▲), and 20% (●).

Compressibility measures the ease (or resistance) of deformation to rupture (as can be seen in Fig. 1) and may be regarded as an indication of fracturability. Therefore, the gels formed at the higher heating temperature and protein concentration were firm, tough, and unfracturable. Elasticities of the gels were similar at all protein concentrations and were reduced by increasing heating temperature.

Effect of glycinin/β-conglycinin ratio on mechanical properties of APP gels. Gels were prepared from the APP containing 56% glycinin (glycinin/β-conglycinin ratio 2.41) and from the low glycinin-APP containing 24% glycinin (glycinin/β-conglycinin ratio 0.88), and evaluated for mechanical properties by using the compression-decompression test. Changes in mechanical properties of the gels are illustrated in Figure 3, where the plots were the same as those in Figure 2. As shown in Figure 3A, rutpure forces of the gels from APP increased with elevated heating temperature ($p<0.05$). This increase becomes large above 93°C. The low glycinin-APP gels gradually increased over the temperature range of 80–96°C. No significant increase was observed above 96°C(0.05 level).

Changes in compression work of the gels were similar to those of rupture force (Fig. 3B). These results, together with those shown in Figure 2 (A and B), indicate again that the gel network structures above and below 93°C are due to the functions of glycinin and β-conglycinin, respectively, considering their denaturation temperature (16). This is also in agreement with other reports in the literature (20). It is worth noting that the gels of low glycinin-APP exhibited higher compression work than the APP gels at heating temperatures below 93°C. This may partly account for previous conflicting observations on the order of gel hardness of β-conglycinin, glycinin, and soy protein isolate (21–23). Figure 3 (A and B) show that the higher the proportion of β-conglycinin (low glycinin-APP), the greater the gel hardness value when the heating temperature is below 93°C. The reverse was observed at temperatures above 93°C. Thus, it is likely that gel hardness, when heating below 93°C, ranks in descending order of β-conglycinin, soy protein, and glycinin, while the order is glycinin, soy protein, and β-conglycinin when heating above 93°C at high ionic strength.

Changes in compressibility of the gels are shown in Figure 3C. Compressibility increased gradually when elevating heating temperature in both the APP and the low glycinin-APP, where the steep rise was observed at 93–96°C. Compressibilities were larger in the APP gels than in the low glycinin-APP gels over the entire temperature range. In other words, low glycinin-APP gels were more fracturable than APP gels. It is worth noting that the APP gels exhibited large compressibilities even when heating at temperature below 93°C, which cannot induce conformational changes in glycinin. This means that glycinin is responsible for high compressibilities of APP gels, regardless of whether glycinin forms network structure or not. Some local and/or partial conformational changes of glycinin molecules may occur below their denaturation temperature, thereby enhancing protein-protein and/or protein-solvent interactions.

Resiliency decreased with increasing heating temperature in both the APP and the low glycinin-APP

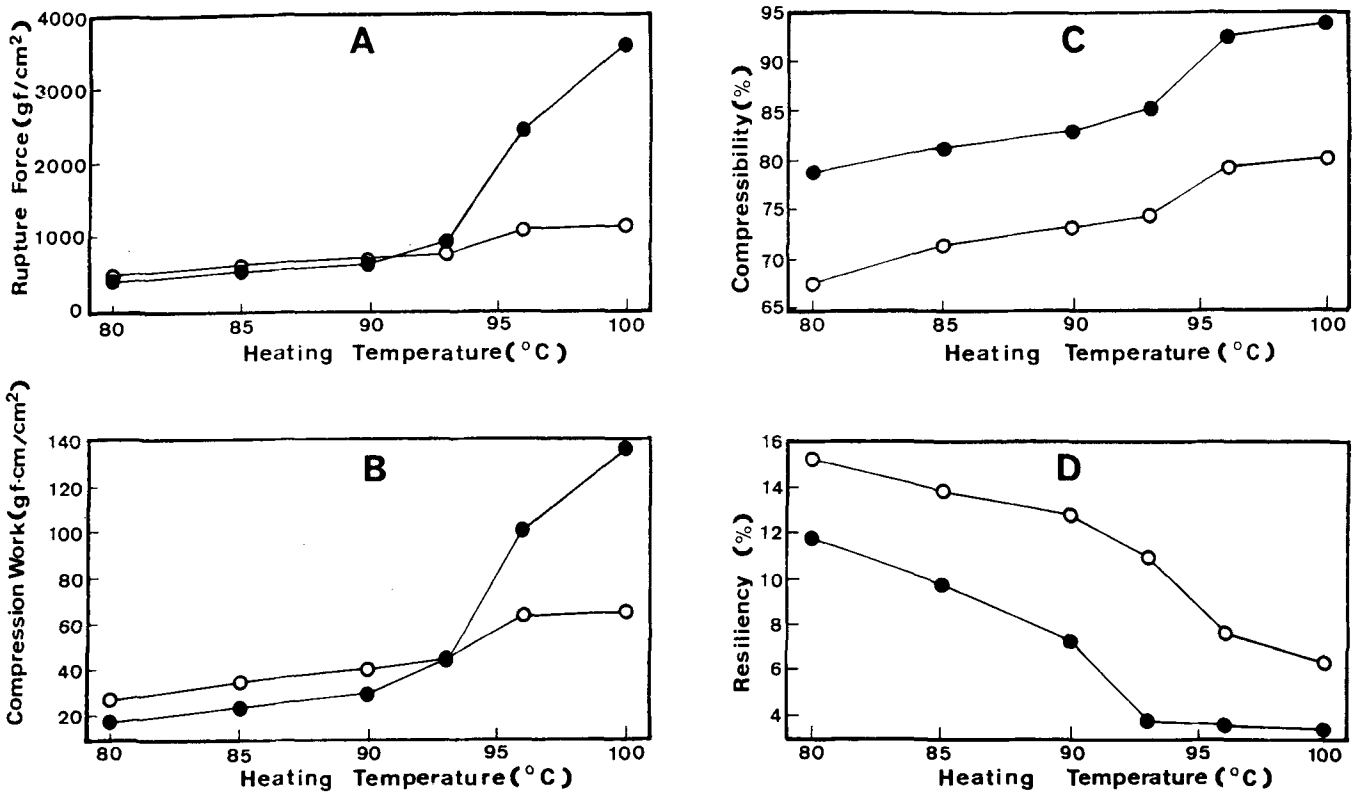

FIG. 3. Effects of heating temperature and glycinin/β-conglycinin ratio on gel properties. A denotes rupture force; B, compression work; C, compressibility; and D, resiliency. Symbols ○ and ● denote the low glycinin-APP and APP gels, respectively. The gels were prepared from the 20% protein solutions.

gels as shown in Figure 3-D. APP gels had less resiliency than low glycinin-APP gels over the entire temperature range. In other words, low glycinin-APP gels were more elastic than were APP gels. This result indicated that β-conglycinin largely contributes to the elasticity of APP gels.

Our results demonstrate that APP gels are more firm and tough, but less elastic than low glycinin-APP gels. However, it has been reported that gels formed from glycinin are more firm and resilient than those formed from β-conglycinin of soybeans (6,8). This discrepancy may be due to the experimental conditions such as heating temperature, the method of protein preparation, and methods used for measuring gel properties.

Evaluation of textural characteristics of APP gels. The force-deformation curves of APP gels formed under various heating conditions appeared to fall into four groups as shown in Figure 4. These four types of curves are slightly different in rupture force, initial slope of the compression curve, and the decompression pattern. Such differences in the force-deformation curves indicate that complete characterization of the properties of gels cannot be accomplished successfully by examining data from the rupture test alone, and suggest that mechanical data at different levels of compression up to rupture are necessary. Thus, the compression-decompression tests at small, medium and large compression levels were carried out to measure the mechanical parameters of the gels at different levels of compression.

The factor loadings after varimax rotation obtained in the procedure of factor analysis are shown in Table 1. Three factors were retained and the cumulative variance by these factors accounted for 97.9% of the total cumulative variance. The factor loadings represent correlations between the factors and the mechanical parameters—the higher the value, the more highly correlated the factor with mechanical properties. Mechanical attributes that load high on factor 1 were compression work and forces of small compression level to rupture. Compressibilities of small compression level to rupture loaded high on factor 2. Resiliency at rupture loaded high on factor 3. As described before, rupture force, compression work, compressibility, and resiliency correspond to hardness, toughness, fracturability, and elasticity, respectively. Therefore, it was construed from the results of factor loadings that factor 1 relates to the hardness and toughness of the gels; factor 2, to the fracturability; and factor 3, to elasticity. The factor scores obtained at the last stage of the factor analysis procedure are shown in Table 2. The gel formed at 100°C from a 20% solution of APP exhibited the highest scores for both factors 1 and 2, which indicates that this gel was the hardest, toughest, and most unfracturable gel. For factor 3, the gel formed at 80°C from a 19% solution of APP exhibited the highest score, which means that this gel was the most elastic. In order to facilitate comparison of gel properties among all the samples, the data in Table 2 were plotted with factors 1, 2, and 3 as Z, X, and Y axes. This choice of factors for the axes provided the best visualization.

Figure 5 shows the diagrammatic representation of the gel samples in three dimensions. The length of the axes correspond to the values of the factor scores, where the

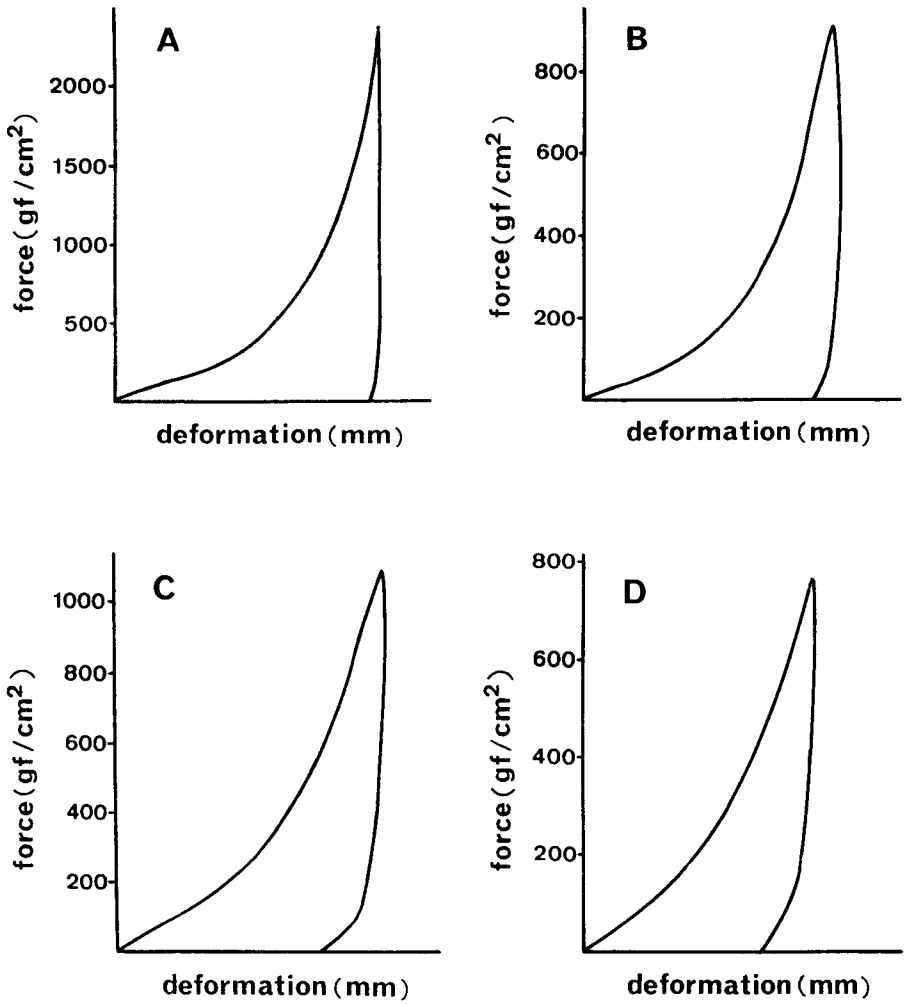

FIG. 4. Force-deformation curves of APP gels from the compression-decompression test at rupture.

TABLE 1

Factor Loadings After Varimax Rotation[a]

Mechanical properties	Factor #1	Factor #2	Factor #3
Compression Work			
Small	0.886*	0.416	−0.195
Medium	0.896*	0.369	−0.237
Large	0.912*	0.298	−0.279
Rupture	0.922*	0.200	−0.308
Resiliency			
Small	−0.415	−0.684	0.460
Medium	−0.469	−0.625	0.594
Large	−0.479	−0.526	0.691
Rupture	−0.240	−0.566	0.758*
Compressibility			
Small	0.291	0.940*	−0.135
Medium	0.345	0.897*	−0.268
Large	0.384	0.846*	−0.346
Rupture	0.382	0.777*	−0.479
Force			
Small	0.925*	0.323	−0.199
Medium	0.925*	0.323	−0.199
Large	0.925*	0.323	−0.199
Rupture	0.925*	0.322	−0.199

[a]Number of factors at varimax rotation: 3. Factor contributions: #1, 7.871; #2, 5.315; and #3, 2.482.
*Factor loadings higher than ±0.722 were marked.

TABLE 2

Factor Scores for APP Gels

Gelling conditions	Factor #1	Factor #2	Factor #3
APP			
18% 80°C	−0.756	0.353	0.556
18% 85°C	−0.783	0.310	0.196
18% 90°C	−0.854	0.269	−0.210
18% 93°C	−0.810	−0.124	−0.960
18% 96°C	−0.669	−0.189	−1.905
18% 100°C	−0.263	0.163	−2.231
19% 80°C	−0.513	0.802	1.486
19% 85°C	−0.408	0.663	1.201
19% 90°C	−0.552	0.535	0.327
19% 93°C	−0.452	0.357	−0.518
19% 96°C	0.214	0.644	−1.191
19% 100°C	0.686	0.880	−0.919
20% 80°C	−0.491	0.515	1.195
20% 85°C	−0.419	0.577	1.088
20% 90°C	−0.452	0.437	0.282
20% 93°C	−0.385	0.530	−0.685
20% 96°C	1.248	1.443	0.002
20% 100°C	2.709	1.637	1.012
Low glycinin-APP			
20% 80°C	0.452	−2.056	0.943
20% 85°C	0.435	−1.797	0.623
20% 90°C	0.492	−1.575	0.801
20% 93°C	0.457	−1.696	0.240
20% 96°C	0.578	−1.307	−0.439
20% 100°C	0.537	−1.371	−0.896

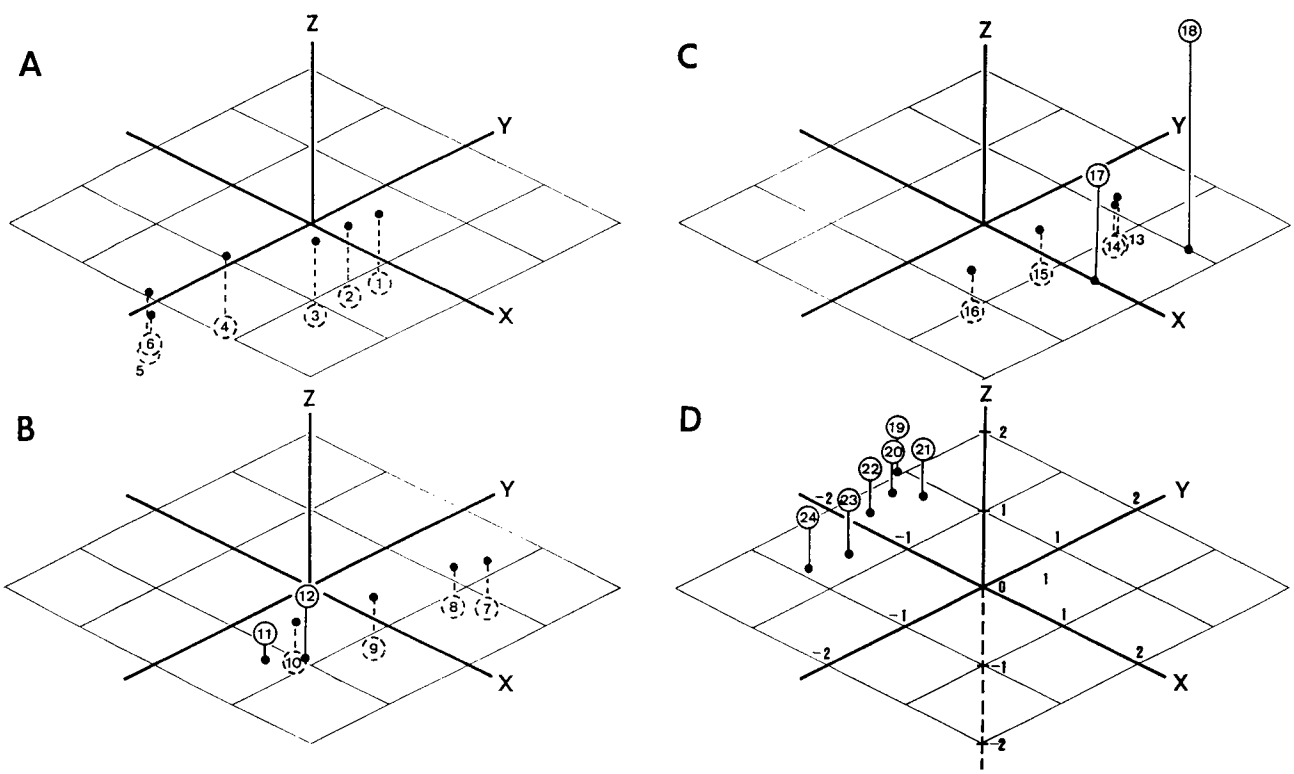

FIG. 5. Three-dimensional representation of gel samples. The coordinate axes of X, Y, and Z represent the factors 2, 3, and 1, respectively. A, B, and C denote the gels from 18, 19, and 20% solutions of the APP, respectively; and D, the gels from the 20% solution of the low glycinin-APP.

scales were represented only in D. The full and dotted circles define the locations of samples in positions on the surface and under the surface of the plane of the X and Y axes, respectively. The gel samples were defined by the number in the circle, 1–6 (A), 7–12 (B), 13–18 (C), and 19–24 (D), each of which defines the heating temperature of 80–100°C shown in Table 2. The properties of the gels and their changes with the gelling conditions were evaluated by their positions in the diagram. Elevating heating temperature shifted the position of the gel samples along the coordinate of factor 3, protein concentration along that of factor 1 and glycinin/β-conglycinin ratio along that of factor 2. From these results, it was demonstrated that the heating temperature had a large effect on elasticity; the protein concentration, on hardness and toughness; and glycinin/β-conglycinin ratio, on fracturability. The results obtained and additional information, which will be gained from this instrumental method, can be useful for texture control and development of soy gels. The thermal behavior of APP which accounts for the diversity of gel properties may be responsible for contributing to the textural quality of foods made from soybeans, such as tofu. However, in these cases, not only the thermal behavior of soy proteins but also the interaction of proteins with other ingredients may influence their texture qualities. The effects of heating conditions on physical properties of soy gel foods have not been investigated systematically yet. Techniques employed in the present study can also be useful for evaluating, discriminating, and characterizating textural properties of composite foods.

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[Received June 27, 1990; accepted February 21, 1991]